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THE ACTION OF VACCINES AND OF CONCENTRATED ANTISTREPTOCOCCUS SERUM IN EXPERI- MENTAL STREPTOCOCCAL ARTHRITIS *

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EXPERIMENTS WITH CONCENTRATED ANTISTREPTOCOCCUS SERUM

Antistreptococcus serum has been employed for therapeutic purposes for several years without altogether satisfactory results. Since there is frequently evidence of the presence in such sera of antibodies, it is possible that low potency may be an important factor in explaining the unsatisfactory results. For this reason it was thought advisable to make some experiments with concentrated serum.

In the preparation of the serum, part of which was done by Dr. L. C. Gatewood, fifty-two strains of streptococci, including viridans and hemolyticus, as well as strains from cases of epidemic sore throat, were injected into horses. The strains were isolated from patients having tonsillitis alone or complicated with acute or chronic arthritis, endocarditis, serositis, nephritis and pyorrhea. The virulence of many strains were tested on rabbits; thirty-four produced acute or chronic arthritis in these animals.

Four horses were used,¹ three being injected with a mixture of various types of streptococci, the fourth receiving only strains from epidemic sore throat. After each strain grew for twenty-four hours on a blood agar slant, it was washed off with sterile normal salt solution, the suspension mixed in a large test tube, a drop or two of chloroform added to this, and the whole heated for sixty minutes at 55 C. After heating tests were made to insure sterility. The suspension was then measured and the required quantity taken for injection. The first doses were equal to the growth of one blood agar slant; subsequently they were increased, depending on the reaction of the horse.

Horses 1, 2 and 3, were given a suspension from thirty-seven strains of streptococci, nineteen of which had produced arthritis in

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1. This work was made possible through the generous cooperation of the Memorial Institute for Infectious Diseases, which gave the facilities for the production of immune serum. I am also indebted to Dr. P. G. Heinemann for advice and assistance in the concentration of the serum.

rabbits. After several doses of dead organisms increasing from one to six slants, Horses 1 and 2 received doses of from one-tenth to one slant of living cocci. The larger doses of these caused local abscesses with such unsatisfactory results that this method of immunization was discarded. It had been previously found by Heinemann and Gatewood² that when doses of from ten to fifteen slants of killed streptococci were injected, the opsonic index of the serum soon reached from 1.8 to 2.5, and he therefore used this quantity of organisms for immunization. The injections were made at intervals of ten days.

Horse 4 was immunized with fifteen strains of the epidemic type of streptococcus. Most of these were isolated from the Chicago epidemic, but organisms from the Boston, Rummelsburg (Germany), and Baltimore epidemics were included in the number. The vaccine was prepared and given as described.

Practically the only reaction noticeable in the horses was a rise in temperature of from 1 to 5 F. after the injection. This was most marked after the earlier injections; especially the first two or three doses of ten slants. Occasionally a horse suffered from stiffness of the joints after an inoculation. Bleedings were made about once a month; 2 gallons were obtained each time. The blood serum was then concentrated and titrated according to the method described by Heinemann and Gatewood.³ They found that the antibodies of anti-streptococcus serum are attached to the globulins similar to the anti-toxins of antidiphtheria serum. Precipitating and dialyzing the globulins by the methods of Gibson and Banzhaf in concentrating antidiphtheria serum, they increased the potency of the concentrate from 3 to 5.6 times the potency of the original serum, as measured by the opsonic index. The opsonic index before concentration ranged from 1.5 to 2.8, after concentration from 5.3 to 13.3. I obtained similar results.

In order to test the efficiency of the serum rabbits were used, since it has been demonstrated by several investigators⁴ that an acute or chronic arthritis is produced readily in these animals. After a few preliminary tests, a streptococcus was found which, in relatively small doses, almost never failed to produce acute arthritis three to five days after an intravenous injection. This streptococcus was isolated from

2. *Jour. Infect. Dis.*, 1912, 10, p. 416.

3. *Ibid.*

4. Walker, *Brit. Med. Jour.*, 1903, I, p. 237; Combs, Miller and Kettle, *Lancet*, 1912, 2, p. 1209; Poynton and Paine, *Lancet*, 1902, 2, 861; Davis, *Jour. Am. Med. Assn.*, 1912, 58, p. 1283; *Jour. Infect. Dis.*, 1912, 10, p. 243; Jackson, *Ibid.*, 1913, 3, p. 364; and others.

the tonsils of a man, aged 42 years, suffering from chronic arthritis of one year's duration. The patient had attacks of febrile exacerbation every four to six weeks, accompanied by swelling of the wrist, shoulder, ankle and knee joints. The heart and kidneys were normal. The tonsils were moderately enlarged but not acutely inflamed. A hemolytic streptococcus (256) was isolated in almost pure culture from the crypts of both tonsils. A dose of this streptococcus of one-twelfth to one-tenth of a twenty-four-hour growth on one blood agar slant, approximately 100,000,000 to 125,000,000 streptococci, on intravenous injection produced joint involvement in 95 per cent. of the rabbits in three to five days. The other 5 per cent. usually died of septicemia within about four days.

The antistreptococcus serum was injected intraperitoneally in amounts varying from 5 to 20 c.c. This route was selected since the absorption would be more rapid than by subcutaneous administration, and the dangers attending intravenous injection could be avoided. Half-grown male rabbits were found to be most suitable. The experiments in rabbits fall into four sets:

1. Rabbits treated with serum after developing acute joint infections, following the injection of streptococci.
2. Those treated with serum immediately following the injection of the streptococci and at intervals until the recovery or death of the animal.
3. Those treated with serum before the injection of the live organisms.
4. Those treated with serum before and again after the injection of streptococci.

I. ANIMALS TREATED WITH SERUM AFTER DEVELOPING ACUTE ARTHRITIS

Of the rabbits treated with serum after the appearance of arthritis but one survived. These rabbits received doses of serum ranging from 5 to 20 c.c. on various days. Brief protocols of some of the experiments follow:

Rabbit 3.—Injected intravenously with 1/10 tube of Streptococcus 256. Left wrist swollen and inflamed on the third day; 10 c.c. of serum intraperitoneally on third day. Death on fourth day.

Rabbit 16.—Injected with 1/10 tube of Streptococcus 256. Left ankle involved on sixth day. Given 20 c.c. serum on seventh day, 15 c.c. on eighth day. Died on ninth day.

Rabbit 17.—Injected with 1/10 tube Streptococcus 256. Left elbow swollen on third day. Injected with 20 c.c. serum on the third, fifth, seventh and eighth days. Died on the ninth day.

Rabbit 23.—Injected with 2 tubes Streptococcus Raven. Left wrist swollen on fourth day, followed by swelling of the right wrist on the fifth day. Given 10 c.c. serum on the fifth and sixth days, and 5 c.c. on the seventh day. Died on the seventh day.

Rabbit 30.—Injected with 1/10 tube Streptococcus 256. Left ankle joint involved on seventh day, followed by swelling of right hip. Serum administered in 6 c.c. doses on the fourth, eighth and tenth days. Rabbit died on fifteenth day. Decreased in weight from 4 lb. 8 oz. to 3 lb. 2 oz.

Rabbit 9.—Injected with 1/8 tube Streptococcus 256. Right wrist affected on third day. Serum administered as follows: 10 c.c. fourth day; 20 c.c. each on fifth, sixth, seventh, eighth, tenth, eleventh, fourteenth and nineteenth days; 15 c.c. on twenty-third day; 7 c.c. on twenty-seventh day; 6 c.c. on twenty-eighth day; 10 c.c. on twenty-ninth day; in all, 220 c.c. of serum. Died on the seventy-fifth day.

Rabbit 10.—Control: Injected with 1/8 tube of Streptococcus 256. Left wrist and knee involved on third day. Death on sixth day.

Rabbit 13.—Control: Injected with 1/10 tube of Streptococcus 256. Right wrist swollen on second day. Died on fifth day.

Rabbit 11.—Control: Injected with 1/8 tube of Streptococcus 256. Left shoulder involved on second day. Killed on sixty-eighth day. The joint was swollen but firm at that time.⁵

Rabbit 9 was the only rabbit in this group that recovered from the acute infection. This animal ran a chronic course which ended eventually with a malformed joint. However, the control, Rabbit 11, passed through a similar clinical course with the involvement of but one joint. In none of the nine rabbits treated with the antistreptococcus serum after the development of acute arthritis were any beneficial results apparent. The course of the disease in these was similar to that of the untreated animals. The antibodies, using the opsonic index as a guide, were not increased and death occurred at a shorter period in treated than in untreated animals.

II. ANIMALS TREATED IMMEDIATELY FOLLOWING THE INJECTION OF THE STREPTOCOCCI AND AT INTERVALS UNTIL THEIR RECOVERY OR DEATH

A brief description of two rabbits will show the action of the serum:

Rabbit 14.—Injected with 1/10 tube of Streptococcus 256, November 26. Given 10 c.c. of serum immediately, and 10 c.c. daily until death on the tenth day. On the third day the tarsal joints of the right forefoot were swollen; on

5. The anatomy of this joint with microphotograph can be found in the article on "Experimental Streptococcal Arthritis in Rabbits," by Jackson, *Jour. Infect. Dis.*, 1913, 12, p. 377.

the fourth day the right knee and left wrist. The joint lesions increased in severity. By the ninth day the rabbit had received 90 c.c. of serum.

Rabbit 18.—Injected with 1/10 tube of Streptococcus 256. Given 10 c.c. of serum immediately and 10 c.c. daily for the first six days. The left wrist became affected on the third day, the left knee and ankle on the fourth day, the right elbow on the fifth. Death occurred on the ninth day.

Rabbit 24.—Control: Injected with 1/10 tube of Streptococcus 256. The left ankle was involved on the second day, the right ankle on the fourth day, both wrists on the sixth day. Death occurred on the tenth day.

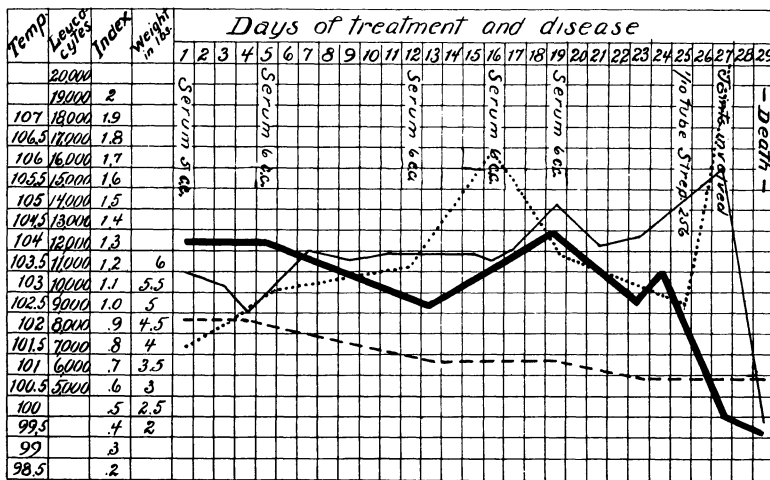


Chart 1.—Prophylactic treatment with serum. Rabbit 35.

Heavy solid line—Opsonic index

Dotted line—Leukocyte count

Fine solid line—Temperature

Fine broken line—Weight curve

Although the treated rabbits received 90 and 60 c.c. of serum, the control animal lived twenty-four hours longer. It might be supposed that these rabbits were overwhelmed by the serum, but the administration of similar amounts to control animals showed nothing but a slight decrease in weight. The rabbits weighed on an average about 2,000 gm. On this basis the amount of serum received daily (10 c.c.) would be equivalent to over 300 c.c. daily for a man weighing 75 kilos (150 pounds). In none of the four rabbits used was serum found in the peritoneal cavity at necropsy. The results in this group are similar to those of the first, and show that the administration of the serum did not increase the resistance of the animals.

III. ANIMALS TREATED WITH SERUM BEFORE THE INJECTION OF THE STREPTOCOCCI

To test the serum as a prophylactic agent against streptococcus infections, it was administered to four rabbits in doses varying from 5 to 20 c.c. at two- and four-day intervals, over a period of two weeks preceding the injection of *Streptococcus* 256. From the experiments it was clearly evident that no protection was afforded by serum, the joints becoming involved usually on the fourth day, with death of the animals by the ninth day. Protocols of two of the rabbits are here given:

Rabbit 34.—February 5, 10, 17, 21 and 24, injected with 5 or 6 c.c. of serum. On March 1, it was injected intravenously with 1/10 tube of *Streptococcus* 256. Right ankle was swollen and tender by the fourth day. Death occurred on the ninth day.

Rabbit 35.—February 5, 10, 17, 21 and 24, given 5 and 6 c.c. of serum. On March 1, was injected with *Streptococcus* 256. Right wrist and left ankle swollen on the fourth day. Death occurred on the fifth day.

Rabbit 37.—Control: Injected with 1/10 tube of *Streptococcus* 256, March 1. Both ankle joints swollen by the fourth day. Death occurred on the ninth day.

IV. ANIMALS TREATED WITH SERUM BEFORE AND AGAIN AFTER THE INJECTION OF THE LIVING STREPTOCOCCI

In this group antistreptococcus serum was administered before and after the injection of the living streptococcus. The four animals treated showed no improvement over the control animals.

Since *Streptococcus* 256 was one of the organisms in the polyvalent vaccine with which the horses were injected in the preparation of the serum, most of these rabbits received an autogenous as well as a polyvalent antistreptococcus serum.

Three streptococci other than 256 were used in producing arthritis to test the efficiency of the serum. The technic was the same as that described, except that the doses of the live organisms were larger, the twenty-four-hour growth on one to three blood agar slants being the usual amount given. Serum was administered in 5 to 20 c.c. doses daily, and at longer intervals. All the animals developed acute arthritis, and died on or before the ninth day.

A chart of a rabbit which had prophylactic injections of serum is given. This chart is selected because the experiments extended over a length of time sufficient to give one a fair idea of the action of the serum on animals, and because the reactions here are typical of those

of other groups. Taking up the opsonins first, we find that in Rabbit 35, the variations are almost identical with those of the control animal, Rabbit 36. Treatment with serum after injection of the living streptococcus showed no changes in opsonins different from those shown on the chart, there always being found a rapid decrease.

There was a gradual loss in weight during the entire period of treatment. This is important, as the control animals during the period preceding injection of the living streptococcus almost always gained in weight. After introduction of the live streptococci, there was in almost all cases a more or less rapid fall in the weight curve. The leukocyte curve was variable; on the whole, there was a gradual increase in the leukocytes during the prophylactic treatment, with a sudden rise in all cases after receiving the living organism. In some cases this went as high as 50,000 leukocytes per cubic millimeter. The temperature curve before the injection of *Streptococcus* 256 is erratic, and is not worth much as a guide to the extent of any serum reaction. There is always a marked rise in temperature after the injection of the live organism reaching nearly 106 F., in some instances with a rapid fall usually immediately before death.

The same concentrated serum, combined with treatment with autogenous vaccines, was employed by Dr. Frank Billings⁶ in a series of cases of chronic arthritides. The serum appeared to heighten the immunizing power of the autogenous vaccines, but anaphylactic reactions were so severe that it had to be discarded. In no case was the serum used as the only treatment. As the serum alone had apparently no immunizing or curative qualities, it was deemed advisable to ascertain the effects of vaccines alone.

EXPERIMENTS WITH STREPTOCOCCAL VACCINES

In the experiments I endeavored to immunize rabbits with repeated doses of homologous streptococcal vaccines, followed by an injection of living streptococci known to produce arthritis in control animals.

The technic was as follows:

The rabbits were injected at intervals of two to four days with five or six doses of vaccine prepared from the organism, which later was injected to produce the arthritis. The injections were made subcutaneously on both sides of the vertebral column. The first dose of vaccine was 500,000,000, and this was gradually increased so that the last one contained 1,000,000,000 to 2,000,000,000 streptococci. Three or four days after the last injection the rabbits were injected intravenously with 1/10 of the twenty-four-hour growth on a blood agar slant

6. *Jour. Am. Med. Assn.*, 1913, 61, p. 821.

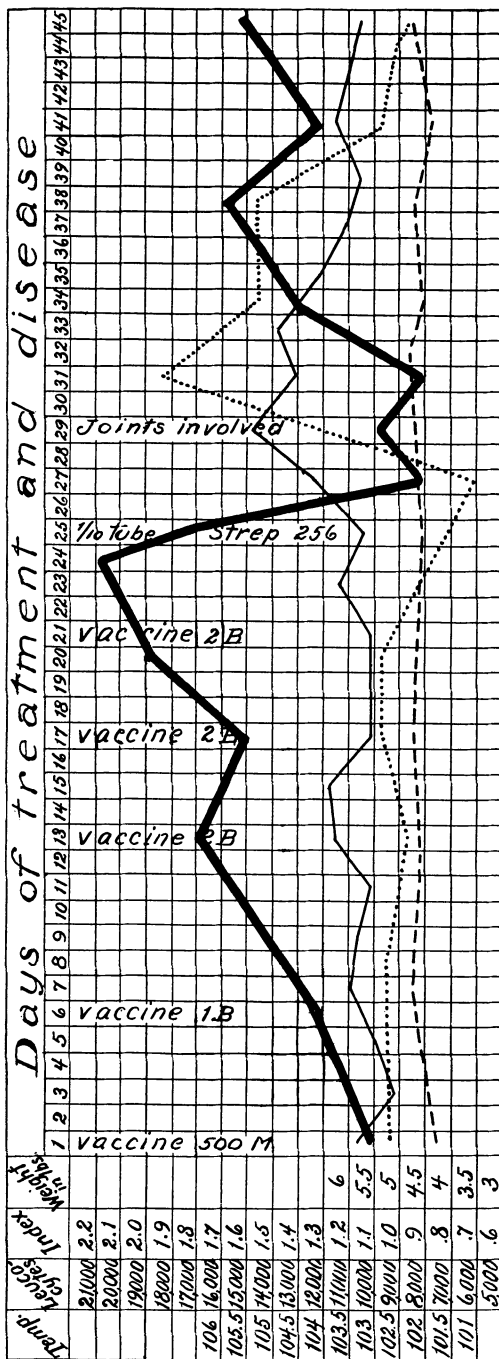


Chart 2.—Prophylactic treatment with vaccine. Rabbit 32. Recovery.

Heavy solid line—Opsonic index
 Fine broken line—Weight curve
 Dotted line—Leukocyte count

of the same organism (*Streptococcus* 256). This amount of the living streptococci (256) had been shown by previous experiments to produce an acute arthritis in 95 per cent. of rabbits within four days, the other 5 per cent. dying of septicemia before that time. Suitable control experiments were made in all cases.

The vaccine was prepared in the following manner: The twenty-four hour growth of the streptococcus on blood agar slants was washed off with sterile normal salt solution, the large clumps broken up, and the suspension standardized in the customary way. It was then heated at 60 C. for one hour and tested as to its sterility, enough phenol added to make a 0.5 per cent. solution and the vaccine preserved in the ice chest. Suitable doses were then taken as required. The vaccine was made each week.

The protocols here presented are typical of the experiments and although some differences appeared the essential details were the same in all cases.

Rabbit 26.—On the first, second, fifth, seventh, tenth and twelfth days injected with 500,000,000 to 2,000,000,000 dead streptococci. One-tenth tube of *Streptococcus* 256 injected intravenously on the fifteenth day. On the eighteenth day 2,000,000,000 vaccine given. Found dead with no development of arthritis on nineteenth day.

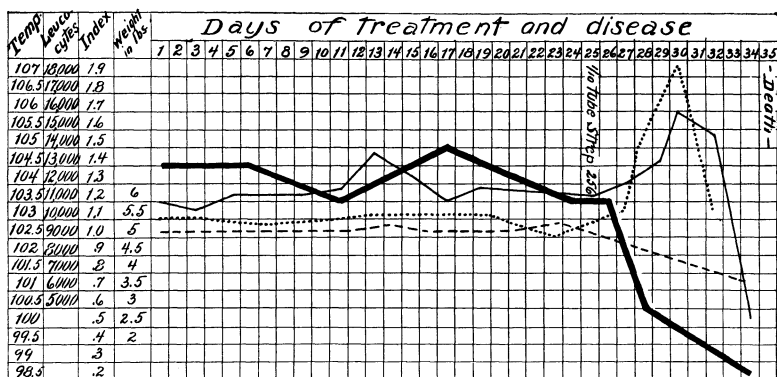


Chart 3.—Control. Rabbit 36.

Heavy solid line—Opsonic index Fine broken line—Weight curve
Fine solid line—Temperature Dotted line—Leukocyte count

Rabbit 32.—On the days shown in Chart 2, given 500,000,000 to 2,000,000,000 dead streptococci. One-tenth tube of living *Streptococcus* 256 injected on the twenty-fourth day. Left shoulder joint swollen on the thirty-first day following a dose of 500,000,000 dead streptococci, and metatarso-phalangeal joint of left foot swollen on the thirty-fourth day, following a dose of 1,000,000,000. Rabbit appears well with slightly swollen joints by thirty-ninth day. Three months later rabbit died of epidemic pneumonia. Found left shoulder joint still enlarged.

Rabbit 33.—On the first, fifth, twelfth, sixteenth and nineteenth days given doses of from 500,000,000 to 2,000,000,000 killed streptococci. One-tenth tube of *Streptococcus* 256 injected on the twenty-fourth day. Left wrist swollen on twenty-ninth day. Swelling disappeared by thirty-ninth day. Three months

later rabbit found dead from epidemic pneumonia. All the joints appear normal at necropsy.

Rabbit 27.—Doses of 500,000,000 to 2,000,000,000 streptococcic vaccine given on first, second, fifth, eighth, tenth and twelfth days. One-tenth tube Streptococcus 256 injected on the fifteenth day. Rabbit showed no symptoms of arthritis. On the fifty-second day injected again with 1/10 tube Streptococcus 256. Aborted two days later and died on the fourth day from general septicemia, with no arthritis.

Rabbit 56.—Vaccine administered in amounts ranging from 500,000,000 to 1,000,000,000 on the first, fourth, eighth, twelfth and sixteenth days. One-tenth tube Streptococcus 256 injected on the twentieth day. The rabbit developed no arthritis. Died of epidemic pneumonia two months later.

Rabbit 25: Control: Injected intravenously with 1/10 tube of Streptococcus 256. Left wrist swollen on the third day, right ankle on the fourth day, right elbow on the fifth day. Died on the seventh day.

Rabbit 36.—Control: Injected intravenously with 1/10 tube Streptococcus 256. Both ankles swollen by the fourth day. Death on the ninth day.

Of fifteen rabbits injected with prophylactic injections of vaccine, five, or 33 per cent., were chloroformed on the first, fourth, ninth, tenth and twelfth days, respectively, to complete a series of pathological studies. Of the ten remaining, two died of the acute infection, one on the fourth day, and the other on the ninth day. The other eight, or 53 per cent. of the total number, lived for various periods of from one to six months, an average of three months, and unfortunately died of the epidemic type of rabbit pneumonia, before the duration of the immunity could be determined. If we limit our figures to those which died from natural causes we have eight out of ten, or almost 80 per cent. of recoveries. These figures are significant when compared with the results in the serum and control groups. One, or 6.6 per cent., of the rabbits treated with serum recovered; one, or 9 per cent., of the control animals recovered. A protection is therefore afforded by the vaccine.

Two rabbits were treated with vaccine both before and after the injection of the living organism. Rabbit 26 received a dose of 2,000,000,000 killed bacteria on the third day after injection with the living culture and was found dead on the fourth day, before the appearance of any arthritis. Rabbit 32 received 500,000,000 on the fourth day, before the appearance of any joint involvement. On the morning of the fifth day the left shoulder was swollen. On the seventh and tenth days, vaccine in 1,000,000,000 doses was given with complete recovery by two months. The large dose of dead organisms in the first case may have brought about the rapid death, and in the

second may have caused the joint involvement. However, we cannot make positive statements from these limited data.

In all cases in the serum control groups, the animals had an acute arthritis or died of septicemia, and all gave symptoms of being sick after receiving the living streptococci. On the contrary, two of the vaccine-treated rabbits did not show any symptoms of being sick and had no joint involvement. Rabbit 27 received a second injection of living streptococci (256) one month after the first, and died in three days from abortion and septicemia. Rabbit 28 was injected twice, the injections being forty days apart; after neither inoculation did arthritis or other symptoms appear. While keeping this animal to test the length of the immunity it died from "rabbit pneumonia."

The average number of joints involved per rabbit in each series is as follows: Controls, 2.4; serum treated, 2.6; and vaccine treated, 1.3. The average number of days after the injection of the living streptococcus that joint inflammations appear is: Control, 2.5; serum treated, 3.5; vaccine treated, 4. The latter figures do not include Rabbits 27 and 28 mentioned in the preceding paragraph. Thus the vaccine not only lengthens the interval of incubation but aids in that a milder form of arthritis is produced, and a smaller number of joints is involved.

We employed as a clinical standard of immunity the production of acute arthritis, since this produces symptoms which can be easily recognized in the living animal. A feature equally interesting, which, however, we did not observe until the animals came to necropsy, was the production of endocarditis. Hemorrhages and vegetations on the mitral valves were most common although the tricuspid and aortic valves and the lining of the left ventricle were quite frequently involved. We found endocarditis in 50 per cent. of the controls, 60 per cent. of those treated with serum, and in only 13.3 per cent. of those treated with vaccine. Since eight of the rabbits lived more than one month, it may be said that these may have had an endocarditis and recovered, but of eight⁷ dying within a period of twelve days after receiving the living cocci, only two showed heart lesions. This is 25 per cent., or less than half that shown in the other groups. Of other pathological changes, Dr. Leila Jackson and Dr. E. R. LeCount⁸ found that acute renal lesions occurred less frequently in the rabbits treated with serum and vaccine than in the controls.

7. Rabbit 27, which died 4 days after receiving the second dose of living streptococcus 256, is included in this number.

8. Tr. Chicago Path. Soc., 1914, 11, p. 112.

The reactions produced in the animals by the vaccine and by the injection of living cocci are shown in the charts. The temperature curves show no sharp changes after the vaccine injections, but in all cases there is a rise from 105 to 106 F. after the injection of living cocci. This falls to practically normal within twelve days. The leukocyte count is also variable. It presents nothing distinctive before the live cocci have been injected, after which there is a sharp rise to 18,000 to 22,000; it then recedes and reaches normal by the eighteenth to twentieth day. An important curve is that of the weight, which in the case of the animals injected with vaccine remained stationary, or showed a gradual increase during the entire period. In the cases of the serum and control rabbits, there was a rapid fall in weight in all instances after the introduction of the living cocci, but this did not occur in the vaccine group except in a few rabbits. As seen on the chart, there is but a very slight decrease in weight, which soon returns to the normal.

The opsonins increase during the prophylactic treatment, fall after the live cocci are injected, but soon rise again above the normal. It would seem, therefore, that the immunity is very intimately associated with, and probably dependent on, the production of opsonins.

SUMMARY

Concentrated antistreptococcus serum was prepared according to the methods employed in preparing concentrated diphtheria antitoxin.

Four series of rabbits were injected with this serum: (a) after the establishment of acute arthritis; (b) immediately after receiving live streptococci; (c) before the injection of the live streptococci; and (d) before and again after receiving the living streptococci.

In none of these groups did the concentrated serum appear to have any preventive or curative effect on the acute arthritis.

Experimental streptococcal arthritis was either prevented by, or ran a much milder course after, prophylactic injections of a homologous streptococcus vaccine.

An immunity was produced, which persisted over forty days in at least one case.

The immunity appeared to be intimately associated with, and probably dependent on, the production of opsonins.

Endocarditis and nephritis were less frequent in the vaccine-treated rabbits than in the controls.